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Determination of apparent digestibility coefficient in fish by stable carbon isotopes

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Abstract

Estimation of the apparent digestibility coefficient ADC(%) of C_3 and C_4 plants in experimental diets for fingerlings of tambaqui (*Colossoma macropomum*, Cuvier, 1818) was calculated by applying chromic oxide (Cr_2O_3) external marker methodology and by a proposed mathematical expression based on the isotopic composition ($\delta^{13}C$). A total of 240 tambaqui fingerlings each weighing ± 48.2 g and measuring ± 9.8 cm were maintained in eight 500-L aquariums specially designed for faeces collection. The ADC(%) of the C_3 and C_4 diets did not differ significantly between the two methods, producing results of 75.6%; 76.2% and 74.4%; 72.8%, respectively. The ADC(%) results obtained by isotopic method presented less variation than by chromic oxide. The proposed mathematical expression for calculating the ADC(%) based on $\delta^{13}C$ values offers an alternative methodology, which can reduce errors and diminish the effort required to collect biological material. However, it is important to note that this method is limited to analysis of diets or food items with distinct isotopic signals.

KEY WORDS: apparent digestibility, C_3 and C_4, fish, stable isotopes

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Introduction

The apparent digestibility coefficient – ADC(%) – is an important parameter not only to evaluate the nutritional quality of a food source, but also to what degree it is assimilated by the animal. It is expressed as a percentage of the quantity of food ingested which is not excreted as faeces (Nacional Research Council – NRC 1993).

The variability in stable carbon isotope values of different food items that make up the diet of an animal can also be used to trace how much of each item is actually digested and absorbed into the tissues of the animal (Jones *et al*. 1979; Bruckental *et al*. 1985; Queiroz 2000; Araujo-Netto, 2002).

Use of stable carbon isotopes is based on the premise that the isotopic ratio ($^{13}C/^{12}C$ or $\delta^{13}C$, expressed in ‰) varies in a predictable fashion in accordance with the cyclical element in nature. In physical, chemical and biological transformations of organic carbon, a naturally occurring discrimination between its isotopes makes it possible to effectively trace the path of that carbon as it passes through an organism (Boutton 1991). Furthermore, a particular food item digested and assimilated by an animal will produce faeces with a different isotopic signal from that which was ingested. Measuring the differences in isotopic values between the ingested food item and the respective faeces, one can estimate how much was digested and absorbed in the tissues of the animal.

To apply stable isotope methodology to diet analysis of an organism, it is necessary that the food sources which make up the diet possess distinct isotopic signals. Plants C_3, C_4 and CAM (Crassulacean Acid Metabolism) are all very distinctive isotopically because of the fractional differences created by diffusion, dissolution and the carboxylation of atmospheric CO_2 (Lajtha & Marshall 1994).

Jones *et al*. (1979) estimated the proportions of C_3 legumes and C_4 grasses ingested by bees through analysis of the isotopic ratios of the two groups of plants. The authors proposed a mathematical expression where the digestibility coefficient could be calculated if the percentage of each plant source consumed was known.

Bruckental *et al*. (1985) compared the $\delta^{13}C$ values of sheep feed and their faeces to determine the digestibility of lucerne

hay (C_3) and corn seed (C_4) in two different feeding regimens: one where feeding was controlled and the other where the sheep could eat freely. The results agreed with those obtained by the traditional total sample method.

In a study comparing the total sample and isotopic composition methods for determining digestibility of fibre in guinea pigs, Queiroz (2000) suggested that the stable carbon isotope method had overestimated the fibre fraction values. However, Araujo-Netto (2002), in comparing the ADC (%) in guinea pigs among total sample, chromium oxide and isotopic composition methods, did not present any conclusive results regarding the for digestibility coefficient of fibre.

Because of inherent difficulties involved in collecting fish faeces to determine the ADC(%), it is imperative that new alternative methodologies are developed which avoid or reduce the current errors. Therefore, the objective of this study was to investigate the potential of the isotopic method as a reliable tool for studies in fish nutrition. We tested the equation modified by Jones *et al.* (1979) to calculate the ADC(%) of experimental diets through comparison of stable carbon isotopes ($\delta^{13}C$) of C_3 and C_4 diets. The results were then compared with those of the traditional method using chromic oxide as an external marker (Burel *et al.* 2000).

Material and methods

Experimental system and faeces collection

The experiment was conducted at the Laboratory of Fish Nutrition in the no ruminates division of the Zootechnical Department, Advanced School of Agriculture 'Luiz de Queiroz', Universidade de São Paulo, Piracicaba, SP, Brazil. A total of 240 tambaqui (*Colossoma macropomum*) fingerlings weighing ±48.2 g and measuring ±9.8 cm were maintained in eight 500-L aquariums, equipped for constant water renewal and with continuous flux aerators. In each aquarium, the fish were housed in rigid 80-L fish traps with an opening at the bottom designed for food insertion and fish collection. The water temperature was maintained at 26 ± 1.5 °C and the water quality parameters (dissolved oxygen, conductivity, pH, alkalinity and nonionized ammonia levels) were monitored daily.

Experimental diets based on C_3 and C_4 plants were formulated by using white rice and soybean meal (C_3) and cornmeal and corn gluten (C_4). Each diet contained 25% of net protein (PB), 3000 kcal kg^{-1} of digestible energy and was supplemented with vitamins and minerals in accordance with the recommendations of Nacional Research Council – NRC (1993) and Vidal Júnior (1995).

The fish were fed with test diets composed of two variations: T-1 = 100% C_3 and T-2 = 50% C_3 + 50% C_4. The diet rations were compressed into 2-mm pellets and administered twice daily *ad libitum* between 8:00 AM 4:00 PM (Table 1).

For faeces collections, four 200-L conically designed plexiglas holding tanks equipped with aerators were outfitted. Partial water changes were done in these tanks and the faeces were pooled within the tank and kept frozen at −20 °C. Faeces collections were only initiated a week after the experimental diets had first been administered, which allowed sufficient time for all gut content from any previous diet to be purged. Each day 2 h after the afternoon rations had been administered, the fish were taken from the feeding tanks in their traps and placed in the holding tanks for 10 h so that their faeces could be collected. The faeces pooled within the tank were centrifuged at 4 °C for 15 min and then stored in the freezer.

Isotopic analysis

The faeces samples were oven-dried at 55 °C and then ground to a fine powder with mortar and pestle. Further processing and isotopic analysis were carried out at the Centro de Energia Nuclear na Agricultura in Piracicaba, São Paulo, Brazil. One milligram of each dry sample was combusted under continuous helium flux in an elemental analyzer

Table 1 Nutritional composition g kg^{-1} and isotopic values of C_3 and C_4 diets

Ingredient	C_3 diet g kg^{-1}	C_4 diet g kg^{-1}
White rice	485	–
Soybean meal	425	–
Soy vegetable oil	85	–
Corn meal	–	601
Corn gluten	–	329
Corn vegetable oil	–	65
Vitamin + mineral supplement	5	5
Composition percentages[1]		
Humidity (%)	73.1	71.4
Crude protein (%)	226.6	259.1
Lipid (%)	91.8	106.7
Ash (%)	28.7	15.7
Carbohydrate (%)	561.8	532.9
Net fibre (%)	18	14.2
ED, kcal kg^{-1} (Nacional Research Coucil – NRC 1993)	3053	3023
Isotopic composition		
$\delta^{13}C$ (‰)	−27.49	−12.34
δ^{15} N (‰)	1.93	3.26

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(Carlo Erba, CHN – 1110) and the carbon isotope ratios determined in a Mass Thermo Finnigan Delta Plus spectrometer. The CO_2 gas produced was analyzed in duplicate with a precision of 0.3%. The carbon isotopic ratio is expressed as delta (δ), in parts per thousand, relative to international standard Peedee Belemnite carbonate as:

$$\delta^{13}C_{\text{sample}}(‰) = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000$$

where, R is the abundance ratio of the heavy to light carbon isotope.

Estimate of apparent digestibility coefficient – ADC(%)

The ADC(%) of the C_3 and C_4 diets was calculated by the following mathematical expression modified by Jones *et al.* (1979):

$$\text{ADC}_{\text{diet}}(\%) = 100 - \left[\left(\frac{\delta^{13}\text{C}_{\text{faeces}(C_3+C_4)} - \delta^{13}\text{C}_4}{\delta^{13}\text{C}_{\text{faeces}C_3} - \delta^{13}\text{C}_4}\right) \times \%\text{C}_3\text{ration}\right]$$

where, $\delta^{13}C_{\text{faeces}}$ is the carbon isotope value of the faeces of fish fed with a T-2 diet (50% C_3 + 50% C_4); $\delta^{13}C_{\text{faecesC3}}$ is the carbon isotope value of the faeces of fish fed with a T-1 diet (100% C_3); and $\delta^{13}C_4$ is the carbon isotope value of a C_4 diet.

The ADC(%) values of C_3 and C_4 diets obtained by isotopic method were compared with the ADC(%) values obtained by chromic oxide (Cr_2O_3) methodology (Burel *et al.* 2000). To obtain the chromic oxide results, the T-1 and T-2 test diets were incorporated with 0.2% of Cr_2O_3. This methodology involved the same fish, procedures, food rations and faeces collections that were used in the application of the isotopic method. However, calculation of the ADC(%) was formulated by the following mathematical expression:

$$\text{ADC}_{\text{diet}}(\%) = 100 \times \left[1 - \left(\frac{\text{Cr}_2\text{O}_3\ \textit{diet}}{\text{Cr}_2\text{O}_3\ \textit{faeces}}\right) \times \left(\frac{\%\text{Plant source}_{\text{faeces}}}{\%\text{Plant source}_{\text{diet}}}\right)\right]$$

The faeces samples were analyzed by the chromic oxide methodology developed by Furukawa & Tsukahara (1966).

Data analysis

The ADC (%) results of the C_3 and C_4 diets by the two methods (carbon isotope analysis and chromic oxide) were analyzed by using ANOVA. The statistical program SAS, version 2.16 (SAS Institute, Cary, NC, USA) was applied for all analyses.

Results

The mathematical expressions proposed by the ADC(%) calculations presented average values that allowed for comparison of results obtained by the different methods. The average ADC(%) values of the C_3 and C_4 diets calculated by carbon stable isotope analysis and external marker chromic oxide tracer were 75.6% and 76.2% (C_3) and 74.4% and 72.8%, respectively (Table 2). The ANOVA calculated that there was no significant difference in the ADC(%) values between the two methods or between diets. Furthermore, the low coefficient variation value encountered (6.7%) demonstrated a high level of statistical precision. However, considerable variation was shown in the ADC(%) values obtained by the chromic oxide method (63.2–83.4% and 58.2–80.4% for C_3 and C_4 diets, respectively), which was not encountered in the values calculated from carbon stable isotope results via the proposed mathematical expression shown in Table 2.

Discussion

The results obtained in this study allowed us to test the applicability of the modified mathematical expression proposed by Jones *et al.* (1979) for calculating the ADC(%) in fish using isotopic values. To verify whether the $\delta^{13}C$ values truly reflected differences in the digestibility of the two source-plant diets, it was necessary to initially test the isotopic compositions of the diet ingredients. Also, to test the validity of the ADC(%) calculations expressed in isotopic values, we utilized the traditional method of chromic oxide tracer (Cr_2O_3), applied mainly in fish digestibility studies. Similarity between results obtained by the two methods

Table 2 Apparent digestibility coefficient ADC ($\mu \pm$ SD) of C_3 and C_4 diets calculated by isotopic method ($\delta^{13}C$) and external marker (Cr_2O_3)

C_3		C_4	
$\delta^{13}C$(%)	Cr_2O_3(%)	$\delta^{13}C$ (%)	Cr_2O_3(%)
74.8	63.2	75.2	58.2
76.4	79.6	73.6	76.7
76.1	83.4	73.9	80.4
75.3	78.3	74.7	75.8
Avg. 75.6 ± 0.7 CV = 6.68%	76.2 ± 8.7	74.4 ± 0.7	72.8 ± 9.9

enabled us to validate the application of the proposed mathematical expression. This was in contrast to the results obtained by Queiroz (2000) who estimated the ADC(%) of the fibre fraction in guinea pig diets comparing total sample and isotopic composition methods. Because of differences observed between the two methods, the author suggested that there had been an overestimation of the digestibility of the fibre fraction in the diet when calculated by isotopic composition. Also, the results of our study differed from the results of Araujo-Netto (2002), which found no difference in ADC(%) values calculated from total sample, chromic oxide tracer and isotopic composition methods.

Validation of the mathematical expression allowed for a comparison of the ADC(%) values between the C_3 and C_4 diet carbon sources. The ADC(%) values for the two plant sources in tambaqui fingerlings were similar to our test conditions. This result suggests that the difference in the isotopic ratios of the diet ingredients does not vary significantly during digestion.

The similarity in the ADC(%) values between the C_3 (rice and soybean meal) and C_4 (cornmeal and corn gluten) diet carbon sources could be due to poor assimilation when the diet is composed solely of these two ingredients. The digestibility of an ingredient for a given species is related to the interactions between the different ingredients which make up the diet (Nacional Research Council – NRC 1993; Lovell 1998).

Analysis of the ADC(%) values utilizing different methods showed less variation within the repetitions of the isotopic method than the chromic oxide method. A larger variation was expected in ADC(%) values utilizing the chromic oxide method because of the possibility of progressive amounts of tracer being leached from the samples during repetitions, and also from analytical error in the laboratory. Similar variation was not encountered in ADC(%) results using the isotopic method as the tracer is the difference in elementary carbon mass of the diet ingredients. The isotopic analysis utilizes a standard sample amount without any intermediary manipulation and the mechanical readout error is $<0.3‰$.

As the results of this study show, utilization of the isotopic method in estimating the ADC(%) can reduce the error and requires less effort in material collection. However, it is important to note that this method is limited to determining ADC(%) values for ingredients or diets with distinct $\delta^{13}C$ signatures.

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